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Hybrid sunflower seed yield, composition and deterioration after chemical desiccation

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Abstract

The impact of chemical desiccation on yield showed contrasting results depending on seed moisture content at the time of application. Its effects on seed deterioration are still unknown and could be modified by seed composition. Objectives were to evaluate the impact of chemical desiccation on: i) hybrid sunflower seed yield and composition at harvest time, ii) seed deterioration during long-term storage and iii) the relationship between seeds deterioration and oil or oleic acid content. Six hybrids including low, mid and high oleic were evaluated in three experiments. Two treatments were applied on female lines at 27-30% seed moisture: (i) spraying with Paraquat and (ii) detaching heads with a knife. Control remained in the field until 10% seed moisture. Seeds were stored during 19 months under room and cold chamber conditions. Yield, number of seeds and hybrid seed composition (thousand seed weight, kernel percentage, oil and acid oleic content) were determined. Seed deterioration during storage was analyzed by germination and vigour. Paraquat advanced harvest by 35-43 days, without affecting yield or seed composition. During storage the germination of Paraquat treatments remained above that of control, without differences between storage conditions, while vigour remained above control only in cold chamber, for low oleic hybrids. Associations between deterioration (germination and vigour) and oil or oleic acid content, were not significant. Desiccation with Paraquat allows advanced harvest without yield losses or modifications in seed composition. The deterioration of desiccated seeds was lower and independent from oil and oleic acid content.

Keywords: Sunflower; Chemical desiccation; Seed productivity; Seed deterioration.

Introduction

Chemical desiccation can be used in hybrid sunflower seed production with the objective to advance the mechanical harvest time. This technique reduces the losses caused by exposure to weather, pathogens, bird attacks and improves harvest operation

(Radić, 2006). Chemical desiccants vary in composition and action mode, with Paraquat (salt of 1, 1'-Dimethyl-4, 4'-bipyridinium) being the chemical most commonly used in grain crops (Bellé et al., 2014).

In oilseed sunflower, physiological maturity (PM) is achieved with 38-40% moisture content (Rondanini et al., 2007). Harvesting before reaching PM could shorten grain-filling duration, reducing seed size or altering its chemical composition (Kappes et al., 2012; Albrecht et al., 2013). Desiccants applications around 50% seed moisture (before PM) either reduced grain yield, seed weight, oil and oleic acid content (Shafiullah et al., 2001; Howatt et al., 2009) or did not affect them (Stahlman et al., 2010). Applications near the PM stage (40-35% seed moisture) may not modify yield or oil content (Howatt et al., 2009, Stahlman et al., 2010) or reduce them without changing seed weight or oil content (Larson et al., 2008). The same controversial responses were observed for applications between 30-14% seed moisture, with either yield or oil content reductions (Liović et al., 2010) or no effects at all (Rana et al., 1989, Stahlman et al., 2010). Thus, the impact of desiccants on seed yield and seed composition showed contrasting results associated to the seed moisture content at the time of application. It is worth mentioning that if the desiccant application is performed with seed moisture below 20%, the harvest stage can be advanced only a few days and so the cost of application is usually unjustified.

Seed quality is a main objective for hybrid sunflower seed production. Oxidative stress and lipid peroxidation are the main causes of deterioration during aging of seeds with high oil content (Bailly et al., 1996; Balešević-Tubić et al., 2005). These biochemical reactions lead to reductions of germination and vigour, affecting field emergency (Kausar et al., 2009) and cause significant economic losses (Mrđa et al., 2010). Since Paraquat is a strong oxidizing agent (Chaneva and Petrova, 2014), it may potentiate oxidative stress and could have damaging effects during seeds storage. After five storage months, sunflower seeds from plants desiccated with Diquat reduced their vigour as desiccant doses increased (Pereira Da Silva, 2011). Recently, it was demonstrated that chemical desiccation with Paraquat improved sunflower seed physiological quality at harvest time (Szemruch et al., 2014). However, its effect on seed quality during long-term storage is still unknown.

During long term postharvest, seed tolerance to peroxidation may vary according to storage conditions (Balešević-Tubić et al., 2005) and seed fatty acid composition (McDonald, 1999). As high oleic acid sunflower has greater oxidative stability (Mourad et al., 2016), the high level of oleic acid could counteract the effects of oxidation (natural or induced by Paraquat), thus minimizing seeds deterioration. The relationship between the rate of deterioration of sunflower seeds and changes in the oil and oleic acid content during storage has not been assessed yet and previous studies analyzed these variables separately. Walters et al. (2005) showed significant changes in oil concentration, acidic profile and free fatty acids content only when sunflower seeds had completely lost their viability, after accelerated ageing. Balešević-Tubić et al. (2007) found significant reductions in germination and vigour of five lines of sunflower, without significant changes in oleic acid or oil content during 12 months storage. Abreu et al. (2013) indicated that germination, vigour and oil percentage of sunflower seeds fell after 12 months, without changes in fatty acid composition. These changes were lower at 10 °C than at 25 °C storage temperature. De Oliveira Lins et al. (2014) found

decreases in vigour, oil and oleic acid percentage, especially after 8 months of storage at 25 °C, but germination was similar for seeds stored at 10 or 25 °C.

Even though the sunflower seed industry is aware of the advantages of chemical desiccation, several topics relating to the hybrid seed production process have not been sufficiently evaluated. This study was aimed to evaluate the impact of chemical desiccation on: i) hybrid sunflower seed yield and composition at harvest time, ii) seed deterioration during long-term storage and iii) the relationship between seeds deterioration and oil or oleic acid content.

Materials and Methods

Crop Management

Three experiments were carried out in Venado Tuerto, Argentina (33º 44' S; 61º 58' W) during two years, on three sowing dates: 25 September 2011 (experiment 1), 31 October 2011 (experiment 2) and 5 November 2012 (experiment 3). The five male sterile genotypes evaluated included four inbreed lines; IL02, IL03, IL04, IL05 and the hybrid IL00 × IL01 (male sterile × maintainer). These were used to produce low, mid and high oleic commercial sunflower hybrids (Table 1). The genotypes G1, G2 and G3 were produced in the three experiments, whereas G3´, G4 and G5 were only produced in experiment 3. G3´and G4 are iso-hybrid for oleic acid (Table 1).

Table 1. Traits of hybrid seeds obtained in experiments 1, 2 and 3.

Code	Female	Male	Crossing type	Oil composition
G1	IL02	IL06	Simple	Low oleic
G2	IL00 X IL01	IL07	Triple	Low oleic
G3	IL10	IL11	Simple	High oleic
G3´	IL03	IL08	Simple	High oleic
G4	IL04	IL08	Simple	Mid oleic
G5	IL05	IL09	Simple	Low oleic

Female lines were planted along eight rows spaced 0.70 m and 5 m long, with a female:male ratio of 5:2. The final number of plants per area (NPA) was 5 plants.m^{-2}. Soil at the experimental site was typical Argiudoll. Plots were managed without nutritional limitations, pests, weeds or diseases. Rainfall was supplemented with drip irrigation to minimise water deficit. Crop phenology was determined according to Schneiter and Miller (1981). There were minimal differences in the crop cycle length among female lines, both at sowing-flowering as flowering-PM period in all experiments (Figure 1). Seed moisture content (m.c.) was determined on samples of 120 g seeds, randomly picked from three plants per plot, by 'low temperature' method (ISTA, 2015) at 103 °C for 17 h ± 1 h.

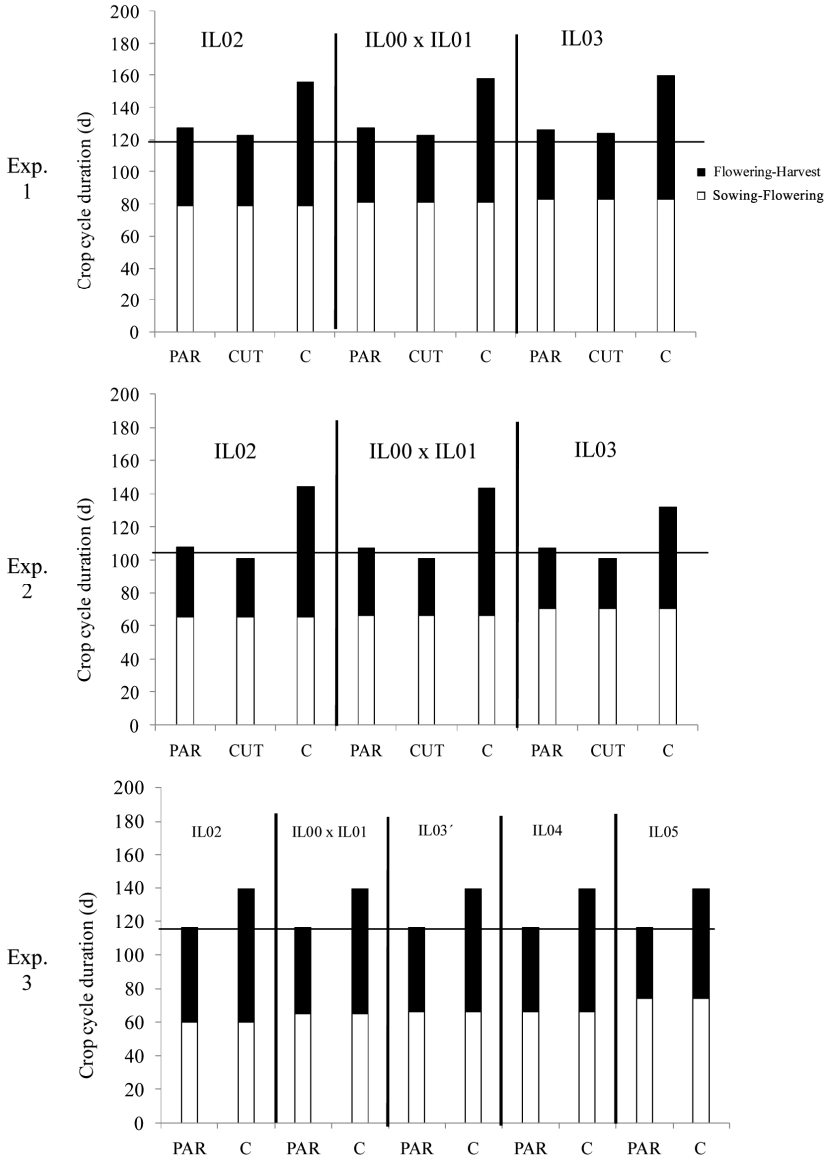

Figure 1. Crop cycle duration (days) for sowing-flowering and flowering-harvest period in five sunflower female lines (IL00 to IL05) sprayed with chemical desiccant Paraquat (PAR) or detached heads (CUT) at 27-30% seed moisture content and control plants (C) that remained in the field up to 10% seed moisture content. Horizontal lines indicate the time of general physiological maturity stage (PM).

Desiccant treatments

Treatments applied on the female lines were: (i) chemical desiccation with Paraquat (PAR) or (ii) physical desiccation detaching heads with a knife (CUT). In PAR treatment the whole plants were sprayed with 2 L. ha^{-1} Paraquat solution in water (1% v/v) using a knapsack sprayer equipped with hollow-cone nozzles with 3 bar pressure, located approximately 20 cm above the crop. Paraquat was sprayed at R8 stage (Schneiter and Miller, 1981), when seeds had reached 27.4 ± 0.5% m.c. in experiment 1, 30.3 ± 0.5% in experiment 2 and 27.2 ± 0.4% in experiment 3 (values are mean ± standard deviation). The heads of PAR were harvested when brown leaves fell to the touch at 4, 7 and 7 days after Paraquat application for experiments 1, 2 and 3

respectively. In this crop stage the seed moisture reached the 25.8 ± 4.7% m.c. in experiment 1, 25.0 ± 2.2% in experiment 2 and 23.1 ± 2.5% in experiment 3. The CUT treatment simulated an anticipated harvest without chemical substances, in order to detect possible toxic and peroxidation effects during seed storage. The heads of CUT treatment were harvested on the same day PAR was applied, when seed moisture was 23.7 ± 1.5% (experiment 1) and 28.4 ± 0.4% (experiment 2). Heads from both treatments (PAR and CUT) were processed in an air-forced fluid bed dryer at room temperature for 48 hours, until seeds reached 7-10% m.c. Untreated control plants remained in the field until seeds reached 10 ± 0.32%, 12 ± 0.48 and 10 ± 0.66%, averaging across genotypes, for experiments 1, 2 and 3, respectively. In experiment 1, desiccant application (PAR) and detaching heads (CUT) allowed advancing the harvest time by about 31 and 35 days for experiment 1 respectively, 32 and 39 days for experiment 2 and 23 days for PAR treatment in experiment 3 (Figure 1).

Heads of ten female plants located along the central two rows of each treatment were threshed mechanically to determine: yield (kg/ha) and number of seeds per area (NSA). Hybrid seed composition was determined by: thousand seed weight (TSW) evaluated from eight subsamples of 100 seeds (ISTA, 2013), kernel percentage (KP) on four subsamples of 50 seeds by manual pericarp removing and oil or oleic acid content by NMR (Spinlock SLK-100, Córdoba Argentina).

Storage treatments and seed deterioration

Seeds from experiments 1 and 2 were stored during 19 months under two environmental conditions: i) at room: 25 ± 5 °C and 30-70% relative humidity (RH) and ii) at cold chamber: 10 ± 2 °C and 60% RH. Seed deterioration was analysed at regular time intervals during storage (1, 5, 9, 13 and 19 months) by germination and vigour on 4 replications of 50 seeds for each treatment (PAR, CUT and Control). Germination was evaluated after pericarp and seed coat removal (SpSc) according to Szemruch et al. (2014) and vigour by electrical conductivity (Braz et al., 2008).

Statistical analysis

Experimental design in each field experiment was a split-plot with two replicates. ANOVA and LSD test were performed ($P<0.05$). Percentage values were transformed using the angular transformation. Correlation analysis was also performed between the germination or vigour (conductivity) and seed composition (oil and oleic acid content). Statistical software was Info stat (www.infostat.com.ar).

Results

Female lines yield and hybrid seed composition

Chemical desiccation did not affect seed yield or NSA in experiments 1 and 2 (Table 2). TSW was not significantly affected by PAR and CUT, with exception of the female line IL03 (high oleic type) in experiment 2, which increased seed weight by 25% with respect to control. Genotypic effects were significant with higher yield in the hybrid female (IL00 × IL01), although treatment × genotype interaction was not detected (Table 2). Seed composition was significantly different among genotypes. G3 showed higher KP, oil and oleic acid content (12%, 8.2% and 43%, respectively) with respect to

G1/G2 average (Table 3). KP was not significantly affected by PAR and CUT, except for G2 in experiment 1 in which there were reductions of 2 and 6% for PAR and CUT, respectively (Table 3). Oil concentration for G3 was slightly lower (2%) in PAR than Control in experiment 1, but it was 2% higher in PAR than Control in experiment 2 (Table 3). Oleic acid was not affected by chemical desiccation in any genotype (Table 3). Similar results were observed in experiment 3. Seed productivity differed among genotypes (1276 to 5609 kg/ha), but chemical desiccation did not affect seed yield for any genotype (Table 4). Seed composition varied among genotypes, with near iso-genic G3' and G4 having the highest oleic acid content (Table 5). PAR did not affect KP, oil, or oleic acid content (Table 5). Overall, no differences between PAR and CUT treatments were found for female lines yield and hybrid seed traits.

Table 2. Number of seeds per area (NSA), thousand seed weight (TSW) and hybrid seed yield in experiment 1 and 2, for three sunflower female lines (IL00 to IL03) under desiccation treatments (PAR = Paraquat; CUT = detaching seed heads). Different lowercase letters indicate significant differences within each column, for a genotype and experiment given. Values for ANOVA tests for experiment (E), genotype (G), desiccation treatments (T) and their interactions are also shown.

Experiment	Genotype	Treatment	NSA (seeds/m^2)	TSW (g)	Yield (Kg/ha)
1	IL02	PAR	3521^a	63^a	2191^a
		CUT	3306^a	63^a	2086^a
		Control	4130^a	63^a	2600^a
	IL00 × IL01	PAR	4400^a	106^a	4636^a
		CUT	4874^a	99^a	4831^a
		Control	4082^a	99^a	4037^a
	IL03	PAR	3666^a	79^a	2868^a
		CUT	3322^a	72^a	2367^a
		Control	4523^a	72^a	3200^a
2	IL02	PAR	1557^a	62^a	966^a
		CUT	2234^a	61^a	1352^a
		Control	1548^a	63^a	966^a
	IL00 × IL01	PAR	4687^a	72^a	3433^a
		CUT	4998^a	67^a	3318^a
		Control	4144^a	83^a	3433^a
	IL03	PAR	4305^a	52^b	2236^a
		CUT	4358^a	50^b	2172^a
		Control	3465^a	65^a	2236^a
E			0.0098	<0.0001	<0.0001
G			<0.0001	<0.0001	<0.0001
T			0.6234	0.0333	0.8754
E× G			0.0001	<0.0001	0.0319
E× T			0.0249	0.0084	0.4951
G × T			0.3036	0.5401	0.0776
E × G × T			0.3277	0.2496	0.0304

Table 3. Kernel percentage (KP) oil content and oleic acid content in experiment 1 and 2, for three sunflower seed hybrids (G1 to G3) under desiccation treatments (PAR = Paraquat; CUT = detaching seed heads). Different lowercase letters indicate significant differences within each column, for a genotype and experiment given. Values for ANOVA tests for experiment (E), genotype (G), desiccation treatments (T) and their interactions are also shown.

Experiment	Genotype	Treatment	KP (%)	Oil (%)	Oleic acid (%)
1	G1	PAR	71[a]	42[a]	51[a]
		CUT	72[a]	42[a]	48[a]
		Control	70[a]	41[a]	50[a]
	G2	PAR	77[ab]	47[a]	48[a]
		CUT	73[b]	46[a]	51[a]
		Control	79[a]	49[a]	50[a]
	G3	PAR	85[a]	49[b]	96[a]
		CUT	86[a]	52[ab]	91[a]
		Control	86[a]	54[a]	93[a]
2	G1	PAR	66[a]	40[a]	55[a]
		CUT	66[a]	40[a]	55[a]
		Control	66[a]	39[a]	56[a]
	G2	PAR	69[a]	48[a]	48[a]
		CUT	71[a]	47[a]	47[a]
		Control	75[a]	46[a]	46[a]
	G3	PAR	81[a]	53[a]	91[a]
		CUT	78[a]	52[ab]	94[a]
		Control	83[a]	50[b]	94[a]
E			<0.0001	0.1350	0.2233
G			<0.0001	<0.0001	<0.0001
T			0.0141	0.7789	0.7798
E× G			0.8811	0.1061	0.0004
E× T			0.3549	0.0019	0.3771
G × T			0.0310	0.2495	0.8861
E × G × T			0.0667	0.0514	0.1027

Table 4. Number of seeds per area (NSA), thousand seed weight (TSW) and hybrid seed yield in experiment 3, for five sunflower female lines (IL00 to IL05) under desiccation treatments (PAR = Paraquat). Different lowercase letters indicate significant differences within each column. Values for ANOVA tests for genotype (G), desiccation treatments (T) and their interaction are also shown.

Genotype	Treatment	NSA (seeds/m^2)	TSW (g)	Yield (kg/ha)
IL02	PAR	3340^a	57^a	1917^a
	Control	3513^a	60^a	2091^a
IL00 × IL01	PAR	5296^a	89^a	4734^a
	Control	6296^a	89^a	5609^a
IL03´	PAR	1858^a	69^a	1276^a
	Control	1780^a	74^a	1317^a
IL04	PAR	1840^a	73^a	1347^a
	Control	2425^a	75^a	1819^a
IL05	PAR	3340^a	62^a	2061^a
	Control	3664^a	62^a	2227^a
G		<0.0001	0.0001	<0.0001
T		0.0879	0.4318	0.1338
G × T		0.5756	0.9369	0.7357

Table 5. Kernel percentage (KP), oil content and oleic acid content in experiment 3 for five sunflower seed hybrids (G1 to G5), under chemical desiccation (PAR = Paraquat). Different lowercase letters indicate significant differences within each column. Values for ANOVA tests for genotype (G), desiccation (T) and their interaction are also shown.

Genotype	Treatment	KP (%)	Oil (%)	Oleic acid (%)
G1	PAR	66^a	34^a	48^a
	Control	63^a	34^a	52^a
G2	PAR	73^a	46^a	44^a
	Control	70^a	46^a	46^a
G3´	PAR	71^a	35^a	89^a
	Control	69^a	35^a	88^a
G4	PAR	65^a	33^a	76^a
	Control	64^a	32^a	75^a
G5	PAR	68^a	37^a	55^a
	Control	66^a	37^a	56^a
G		0.1310	<0.0001	<0.0001
T		0.2683	0.8869	0.3339
G × T		0.9932	0.9777	0.4750

Deterioration of desiccated seeds

There were no significant differences between PAR and CUT in germination and vigour during storage (Figure 2). The germination of seeds from PAR treatments remained above control during storage, without differences between room and cold

chamber conditions (Figure 3 a-d). Low oleic hybrids (G1 and G2) showed levels of germination higher than minimum commercial value of 85% (Figure 3). The high-oleic hybrid had low germination (< 85%) during all storage period (Figure 3 e, f).

The vigour of seeds from PAR treatments remained above the control only in cold chamber storage for low oleic hybrids (G1 and G2) (Figure 4 a-d). Across all the storage period, the high-oleic hybrid (G3) had more than 70 $\mu S.cm^{-1}.g^{-1}$ (Figure 4), indicating less vigour. This value was above the threshold for high vigor sunflower seeds (Szemruch et al., 2015). In this genotype (G3) and controls of low oleic hybrids differences in the vigour between storage conditions were not detected.

In most experiments and genotypes, correlation between seed composition (oil and oleic acid content) and seed quality (germination and vigour) were not significant, exhibiting low coefficients (≤ 0.26). During storage, the changes in oil content (ranging 35-55%) (Figure 5 a, b) or oleic acid content (ranging 30-90%) (Figure 5 c, d) were not related to germination and vigour evolution of desiccated sunflower seeds.

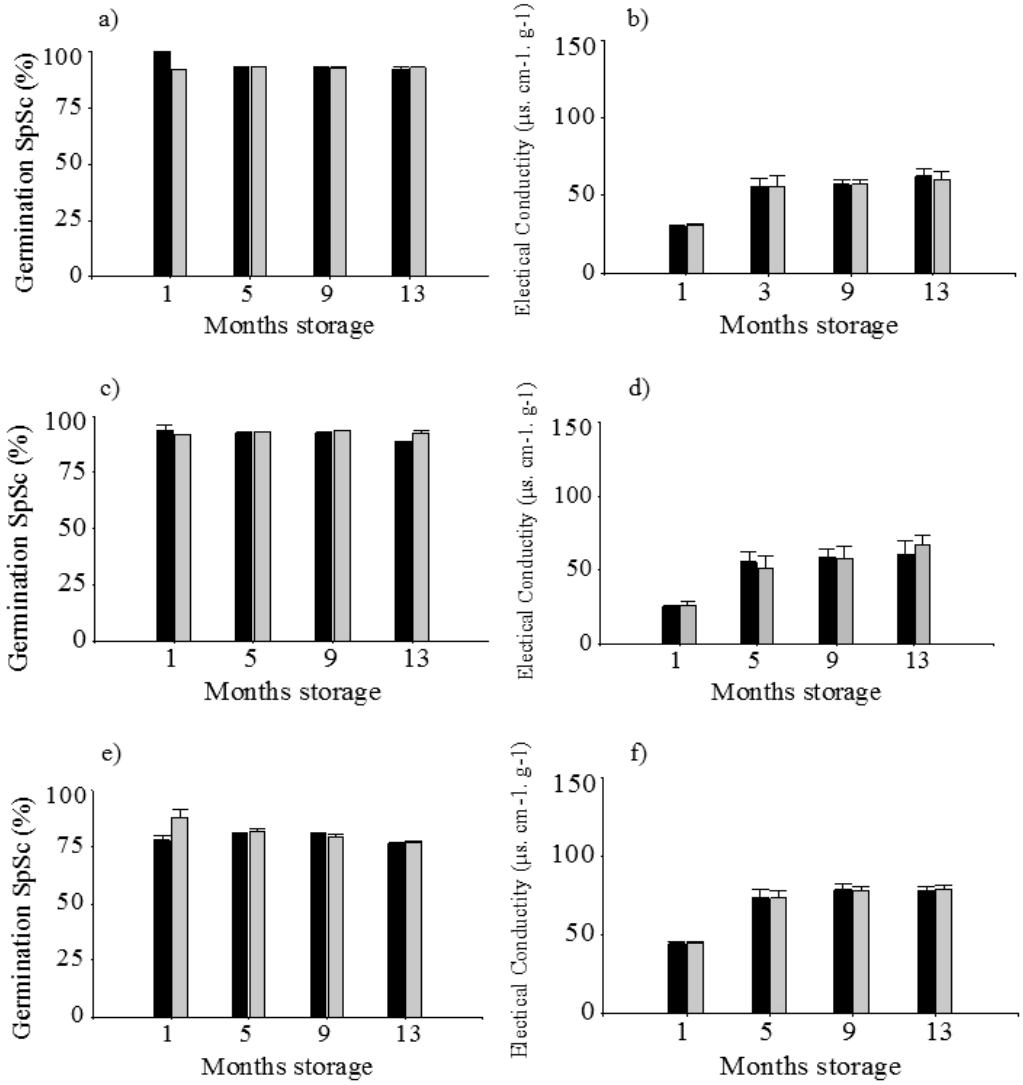

Figure 2. Evolution of germination by SpSc (a, c, e) and vigour by Electrical conductivity during storage for G1 (a, b), G2 (c, d) and G3 (e, f) hybrids under chemical desiccation with Paraquat (gray symbols) and CUT (black symbols) treatments in experiments 1. Values represent average between room and cold chamber conditions. SpSc = germination after pericarp and seed coat removal. Bars are ± 1 standard error.

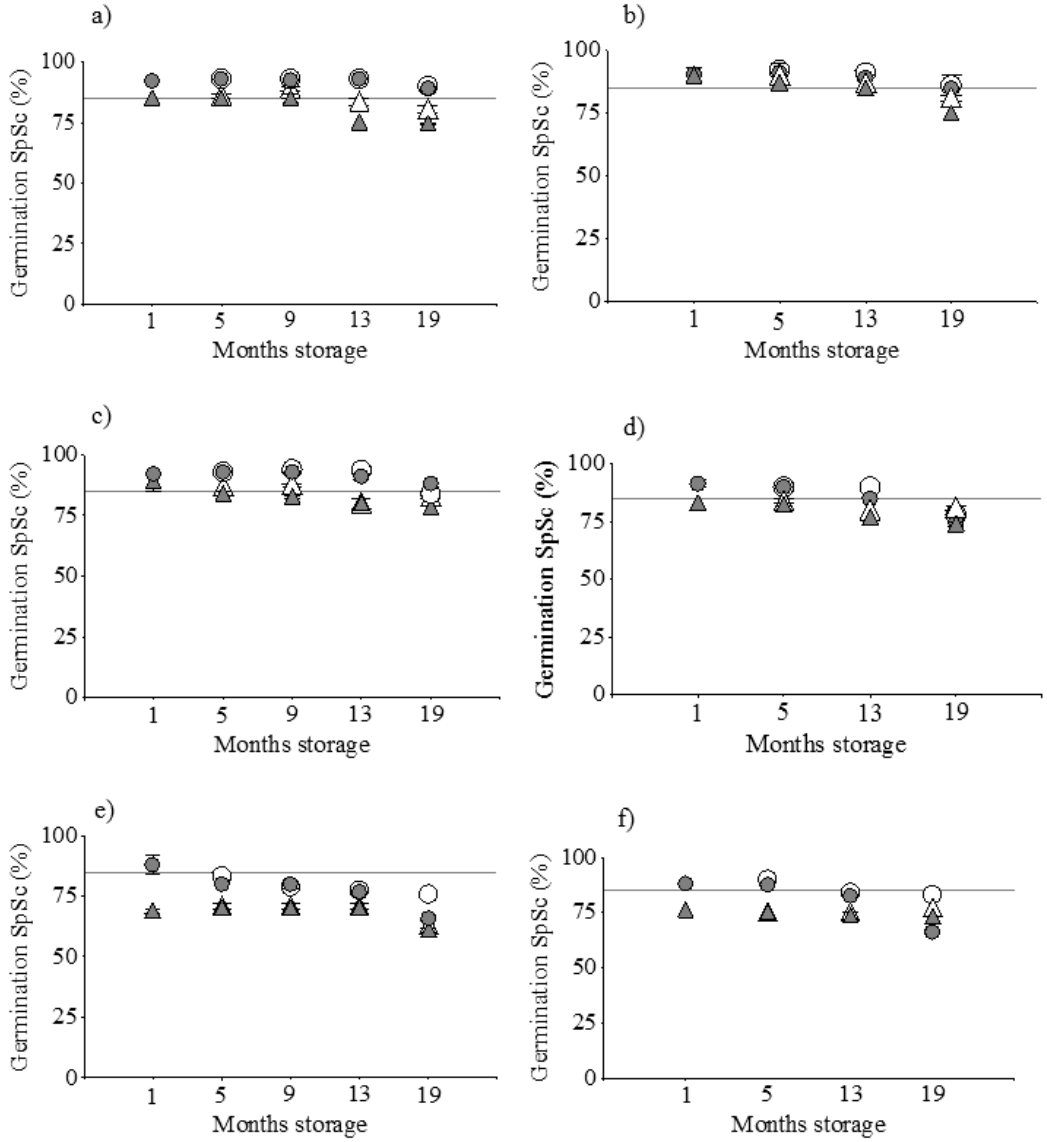

Figure 3. Evolution of germination seeds by SpSc during storage for low oleic hybrids G1 (a, b), G2 (c, d) and high oleic hybrids G3 (e, f) under room (gray symbols) or cold chamber conditions (white symbols) after chemical desiccation with Paraquat (circles) or control (triangles) treatments in experiments 1 (a, c, e) and 2 (b, d, f). Horizontal lines indicate national commercial minimum germination (85%). Bars are ± 1 standard error.

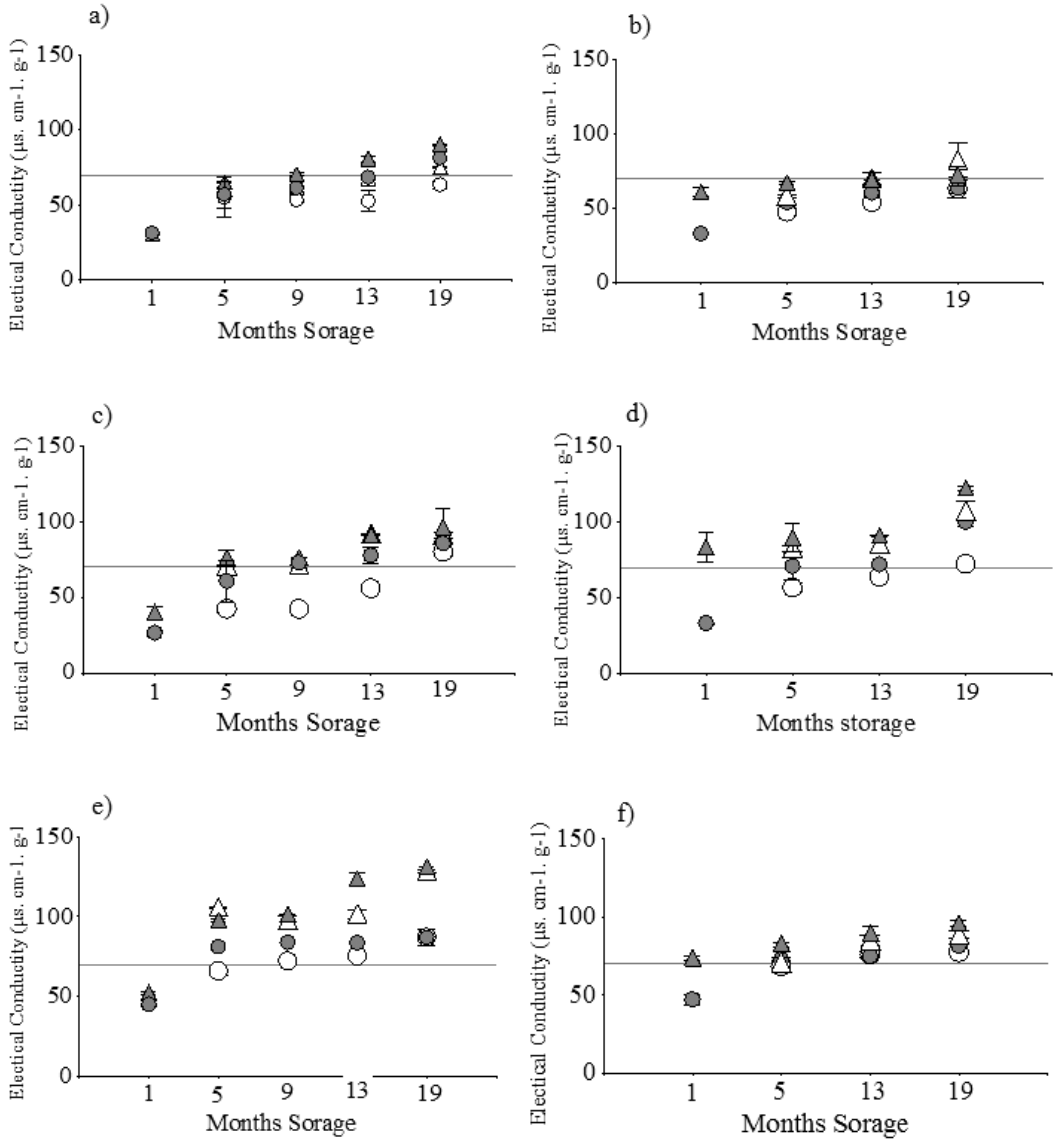

Figure 4. Evolution of seeds vigour by Electrical conductivity during storage for low oleic hybrids G1 (a, b), G2 (c, d) and high oleic hybrids G3 (e, f) under room (gray symbols) or cold chamber conditions (white symbols) conditions under chemical desiccation with Paraquat (circles) and control (triangles) treatments in experiments 1 (a, c, e) and 2 (b, d, f). Horizontal lines indicate Value of electrical conductivity threshold for high vigor seed according to Szemruch et al. (2015). Bars are ± 1 standard error.

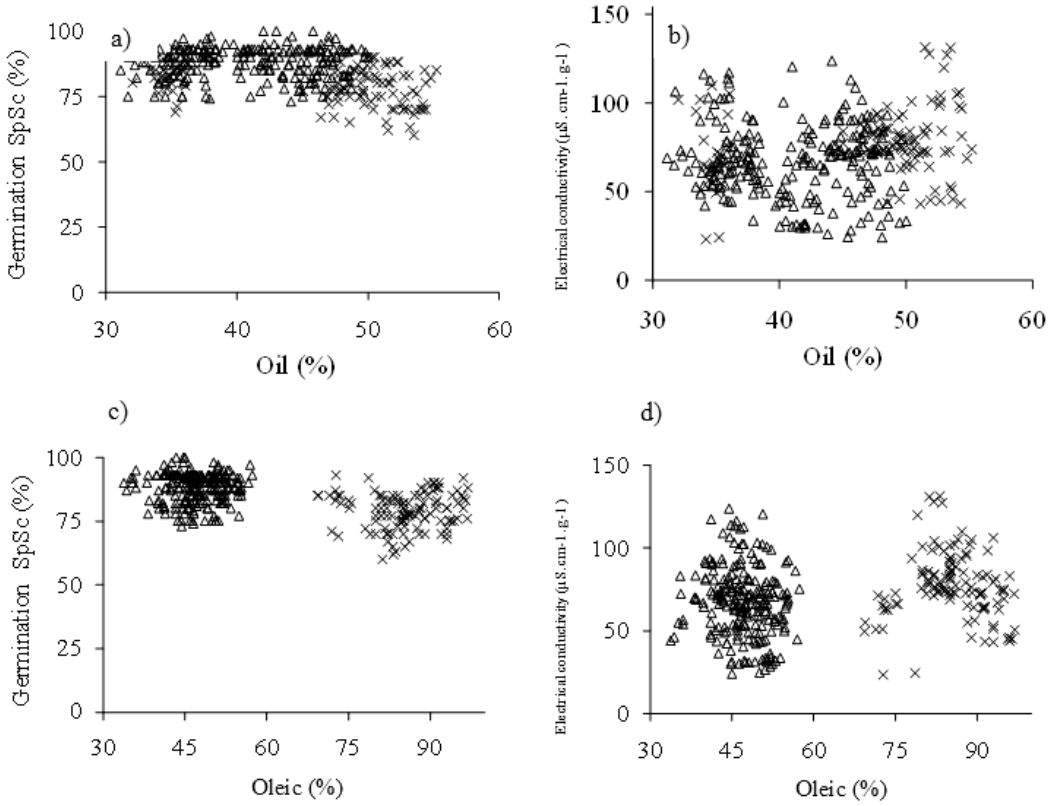

Figure 5. Associations between germination by SpSc (a, c) and vigour by electrical conductivity (b, d) regarding to oil (a, b) and oleic acid content (c, d) for low oleic hybrids G1, G2 (triangles) and high oleic hybrids G3 (crosses) during storage. Data (n= 324) are from experiments 1 and 2 including all genotypes, harvest treatments (PAR-CUT-Control) and long term storage treatments (room-cold chamber; 1-5-9-13-19 months).

Discussion

In the range of 27-30% seed moisture content, chemical desiccation with Paraquat did not affect yield, oil and oleic acid content or seed kernel size, as had previously been observed (Rana et al., 1989; Howatt et al., 2009; Larson et al., 2008; Stahlman et al., 2010). According to Stahlman et al. (2010) the greatest benefit of desiccation could be observed when performed as soon as possible after PM, without sacrificing yield. Nevertheless, applications very close to PM can represent a risk because of the difficulty in accurately determining seed moisture level in sunflower (Kleingartner, 2010). For this reason, in hybrid sunflower seed production, desiccant application in the range of 27-30% moisture seed content ensures a sufficiently early harvest without risk of yield loss or altered seed composition, as demonstrated in this work.

The absence of detrimental effects of PAR treatments in germination and vigour during storage would discard phytotoxic effects on seed metabolism and indicate that Paraquat did not exert detrimental effects on sunflower seeds production. This fact is in line with the idea that the product is safe to be used in seed industry, as has also been demonstrated for soybean, bean and canola (Greven et al., 2004; Marchiori et al., 2002; Bülow and Cruz-Silva, 2012). Germination results in both storage conditions are consistent with De Oliveira Lins et al. (2014) who also found no differences in germination when seeds were stored at 10 and 25 °C. The benefits of cold chamber

storage in seed vigour match those found by Abreu et al. (2013), Lima et al. (2014) and De Oliveira Lins et al. (2014). Low temperatures minimize chemical reactions in general (Bonner, 2008) and oxidation in particular (Balešević-Tubić et al., 2005) delaying the deteriorative process and extending the viability of the seeds (Mohammadi et al., 2011). As shown by these results, the storage in cold chamber benefits the maintenance of sunflower seeds quality, only if they present high initial vigour. When starting with seeds with low vigour (as in controls and high oleic genotypes), storage in cold chamber does not report clear benefits.

There were no changes in sunflower seed deterioration tolerance due to changes in oil concentration or composition, unlike results by Balešević-Tubić et al. (2005) and Ghasemnezhad and Honermeier (2007). The changes in physiological quality of sunflower seeds desiccated with PAR cannot be attributed to oleic acid content, discarding the hypothesis of a slower deterioration in seeds with high levels of this acid. These trends are consistent with Walters et al. (2005) who only detected significant changes in oil and free fatty acids content, when sunflower seeds had completely lost their viability. In a similar way, Ravber et al. (2015) only found significant increases in free fatty acids concentration after sunflower seeds were subjected to extreme temperatures (> 220 °C), noting that oil is the most stable component in sunflower seeds. Morscher et al. (2015) found the viability loss in sunflower is more associated with protein oxidation than with lipid oxidation. During sunflower seeds post maturation or drying, Lehner et al. (2006) found no changes in oil content or composition, but they found major changes in the physical properties of the reserve lipids. These findings show that changes in the mobility of the lipid molecules (transition from the "glass" to "rubber" state) and their reorganization would play a vital role in the acquisition of germination capacity and the potential quality of sunflower seeds. Same considerations could apply to the prolonged storage period and suggest that sunflower seeds deterioration is probably associated with changes in the physical properties of lipid reserves rather than with changes in the oil concentration or composition.

Conclusion

Chemical desiccation with Paraquat applied in the range of 27-30% seed moisture advanced harvest operation by 23-43 days, without yield losses or modifications in seed size or composition. During long term storage, the deterioration of sunflower desiccated seeds was lower and independent from oil and oleic acid content.

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